Role of COX-2 in liver and heart mitochondrial function

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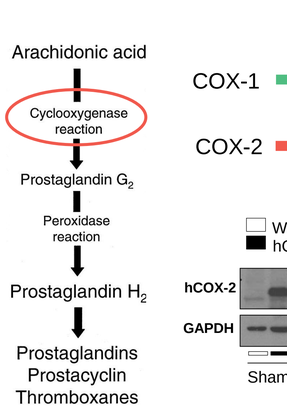
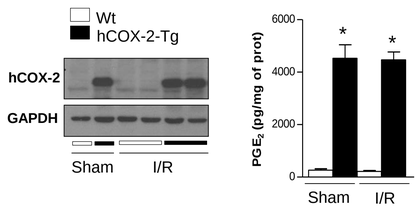
Liver and heart are two principal organs in the body. Although their physiology is extremely different, both tissues have in common that adult hepatocytes and cardiomyocytes fail to induce COX-2 expression, the key enzyme in the synthesis of prostaglandins, regardless of the pro-inflammatory factors used. In both cell types, COX-2 expression is restricted to those situations in which dedifferentiation or proliferation occur. In parallel studies, using mice genetically modified to selectively express hCOX-2 in hepatocytes [1] and cardiomyocytes [2], we have demonstrated an increased tolerance to ischemia-reperfusion injury (IRI) with an increased functional recovery, a diminished cellular necrosis and less inflammation. It is known that mitochondria have a major role in IRI damage by increasing oxidative stress, decoupling metabolic state and inducing apoptosis. In this work, we analyse different aspects in order to characterize the impact of COX-2 in mitochondrial function.

Introduction and previous results

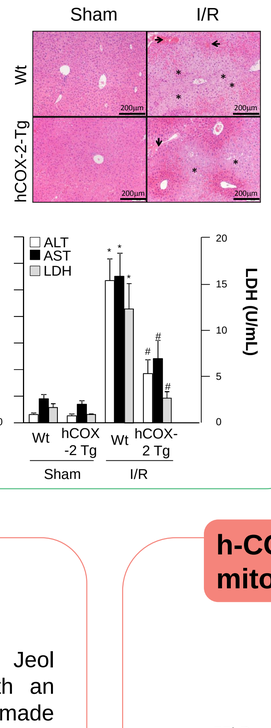


COX-1 → Constitutive expression (homeostasis)

COX-2 → Inducible expression under determined stimuli (adaptation)



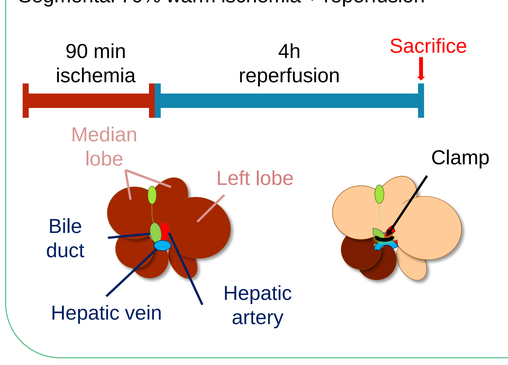
hCOX-2-Tg mice are protected against Ischemia/Reperfusion-induced liver damage [1]

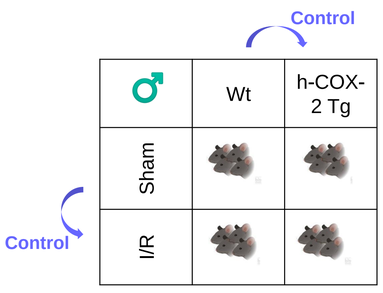


Experimental design

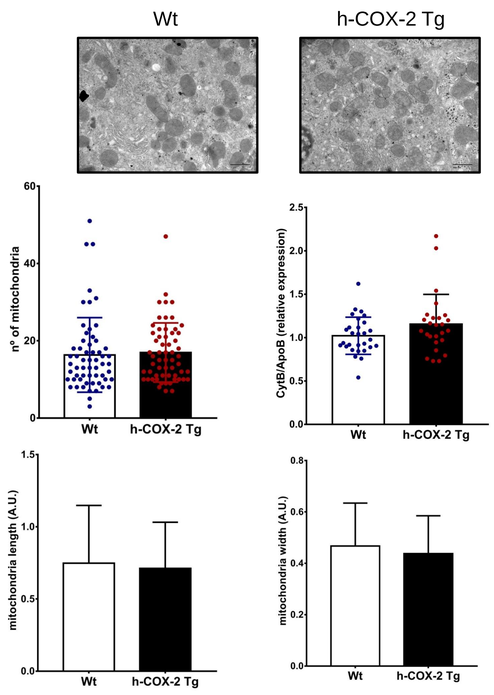
B6D2RccHsd-Tg (APOE-PTGS2)4/Upme → Constitutive overexpression of the human-COX-2 in hepatocytes

Segmental 70% warm ischemia + reperfusion

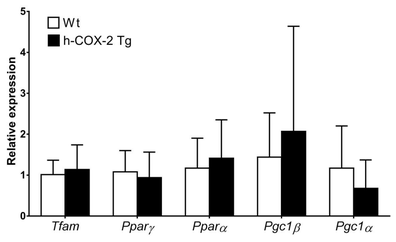




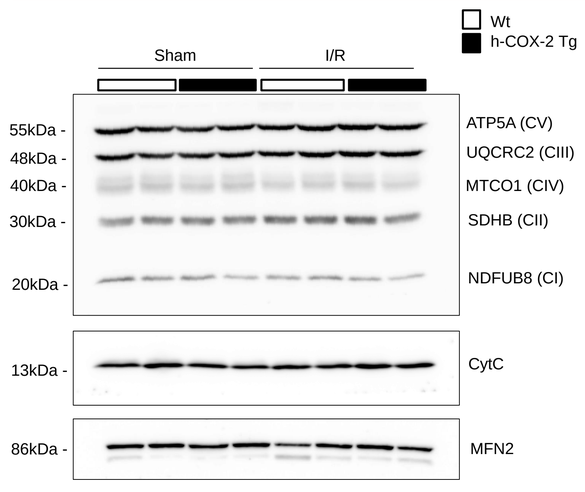
Mitochondria morphology and number are not altered because of COX-2 overexpression

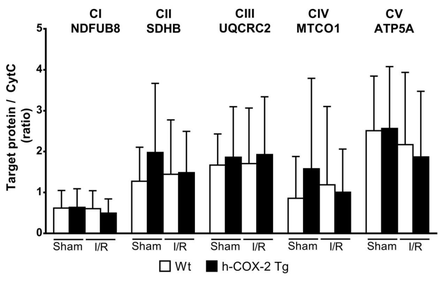


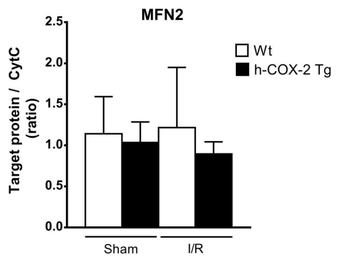
TEM images were obtained by a Jeol JEM1010 microscope at 60 kV, with an x4000 magnification. 10 images were made for each animal (N=5). Mitochondrial-to-nuclear ratios were determined in liver from Wt (N=29) and hCOX-2 Tg (N=26) mice after I/R by real-time PCR. The copy number ratio (mtCytB/ApoB) was calculated using the ΔCt method. Biogenesis-related genes expressions were determined by real-time PCR (N=11) and the fold-increase was calculated using the ΔCt method.



h-COX-2 Tg mitochondria don't show differences in mitochondria proteins

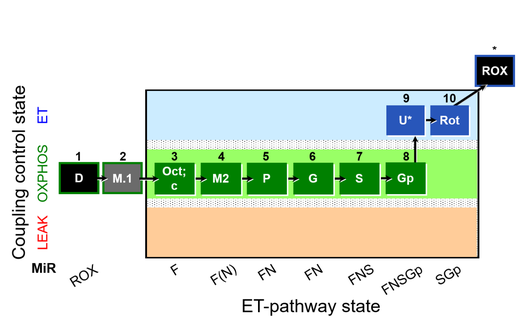


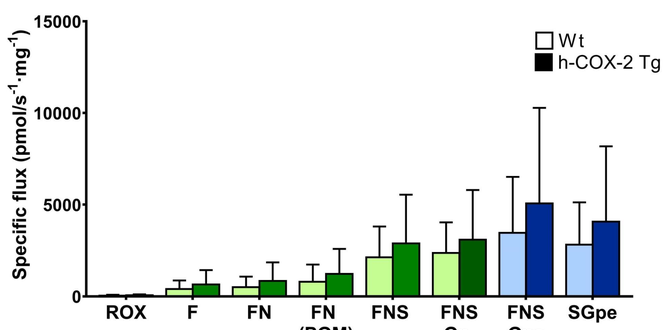


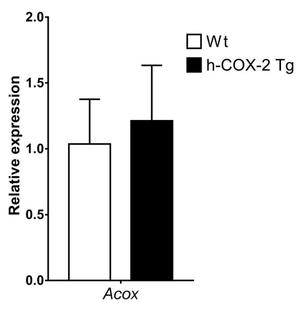


Mitochondria were isolated from Wt and h-COX-2 Tg livers (N=10-13) by differential centrifugations and separated on a 10% polyacrylamide-SDS electrophoresis gel (SDS-PAGE). Blots were incubated with OXPHOS Antibody (Abcam), CytC (Invitrogen) and MFN2 (Abcam). No significant differences are revealed comparing Wt-Tg, neither Sham-IR.

h-COX-2 Tg derived mitochondria show higher respiration because of an increase in β-oxidation

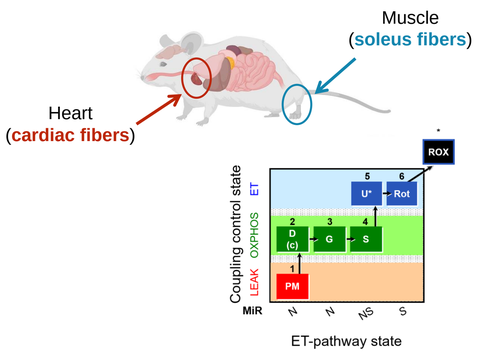


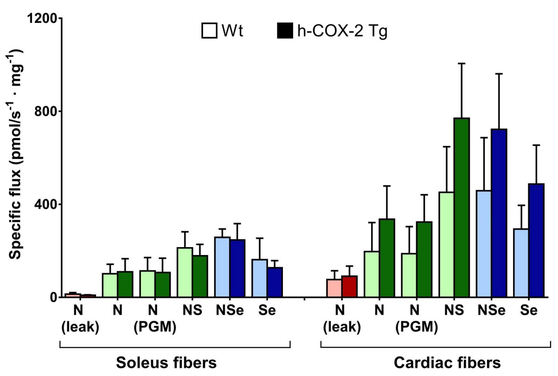




High-resolution respirometry was performed with Wt and h-COX-2 Tg derived mitochondria after I/R with the Oroboros Instrument (O2k, Oroboros, Austria). OXPHOS and ETS capacity were measured with the SUIT RP2 protocol. h-COX-2 Tg-derived mitochondria appears to have a discrete higher flux than Wt-derived (N=8), apparently because of a higher contribution of fatty-acid oxidation. The expression of *Acox* gene, higher in h-COX-2 Tg samples, correlates with the respirometry results.

COX-2 overexpression in liver has an "endocrine" effect in heart fibers

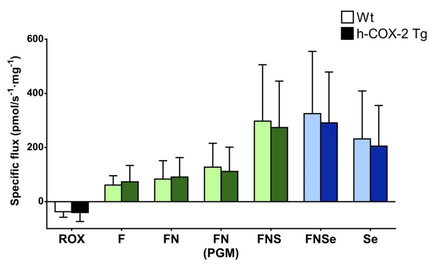


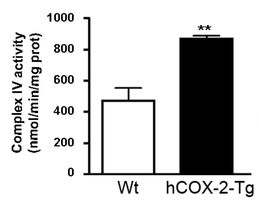


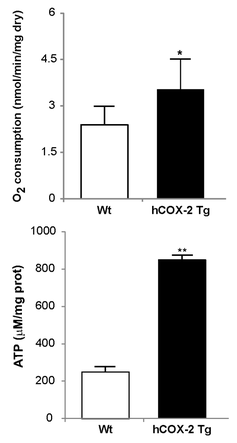
High-resolution respirometry was assessed in soleus and cardiac fibres of Wt and h-COX-2 Tg mice (COX-2 overexpression in liver). In soleus fibres there were no differences, but, surprisingly, heart fibres in h-COX-2 Tg mice had higher respiration, compared to Wt. This result reveals a kind of endocrine effect of COX-2 overexpression in liver, by releasing some substances, prostaglandins or others, that may have an effect away from the tissue where they are synthetized.

COX-2 modulates the complex IV of the respiratory chain in cardiomyocytes

B6D2RccHsd-Tg (α-MHC-PTGS2)4/Upme → Constitutive overexpression of the human-COX-2 in cardiomyocytes







COX-2 overexpression in cardiomyocytes has no effect in basal mitochondrial respiration but there is an increment in CIV activity. CIV activity was measured with a colorimetric assay. ATP amount, measured with a kit (ATP Bioluminescence Assay Kit HS II (Roche)), is higher in h-COX-2 Tg hearts, as well as oxygen consumption (Oxy 1, Hansatech). These results correlate with the effect of the overexpression of COX-2 in hepatocyte, supporting the idea of a paracrine effect of COX-2 products in the organism.

Data represent the mean values ± SE; * p<0.01, ** p<0.05 hCOX-2 Tg mice *vs.* Wt

Conclusions

- COX-2 overexpression has no effect in mitochondrial biogenesis, neither mitochondrial morphology or ETC proteins expression in liver.
- Higher respiration of h-COX-2 Tg-derived mitochondria could be explained by an increase in β-oxidation.
- Products of the COX-2 pathway are acting in an "endocrine" way in heart fibers.
- CIV activity is increased when overexpressing COX-2 in heart, but not OXPHOS respiration.

References

[1] Motiño O, Francés DE, Casanova N, Fuertes-Agudo M, Cucarella C, Flores JM, Vallejo-Cremades MT, Olmedilla L, Pérez-Peña J, Bañares R Boscá L, Casado M, Martín-Sanz P (2019) Protective Role of Hepatocyte Cyclooxygenase-2 Expression Against Liver Ischemia–Reperfusion Injury in Mice. Hepatology, 0,0:1–16.
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